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Determination of fat in live farmed Atlantic salmon using non-invasive NIR techniques

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Abstract: Non-destructive near-infrared (NIR) measurements were performed on 100 live, anaesthetised farmed Atlantic salmon, whole weight 1–11 kg, using two different NIR instruments: a grating monochromator instrument equipped with a fibre optic interactance probe, and a diode array instrument measuring diffuse reflectance in a non-contact mode. Crude fat content was determined using partial least squares (PLS) regression. Full cross-validation was used to evaluate the performance of the calibration models, expressed as the root mean square error of prediction (RMSEP). For the fibre optic instrument the wavelength range from 800 to 1098 nm resulted in a correlation coefficient of 0.90 and an RMSEP equal to 14 g kg^{-1} fat. The diode array instrument using the wavelength range from 900 to 1700 nm gave results of the same accuracy. The measurement times were 21 and 3 s respectively. It is concluded that either instrument could be used to determine the crude fat content in live Atlantic salmon, with good accuracy.

Keywords: NIR; fibre optic probe; diode array; live salmon; fat

INTRODUCTION

One of the reasons for the success of Norwegian salmon farming is the result of a 30 year continuous breeding programme and the concomitant improvement in the chemical composition of the feed. Since 1972 the fully grown weight of farmed Atlantic salmon (*Salmo salar*) has increased by 10–12% on average in every generation and is now almost 80% greater than that of the original wild salmon. During the 1970s the upper limit for dietary fat in salmon feed was 170–180 g kg^{-1} feed.[1] Today, new production technology using extrusion and coating has made it possible to increase the fat content in the feed,[2] which is now usually around 400 g kg^{-1}. This increased fat content, however, has resulted in a higher fat content in the fillet of the salmon.[2–5] Different markets have different preferences for fat content and this has raised the need for non-destructive measurement of fat in salmon.

It has been shown that it is possible to measure fat and moisture in whole post-rigor salmon by near-infrared (NIR) diffuse spectroscopy.[6–9] Measurements on live salmon would make it possible to monitor the feeding regime and also to determine the fat content before slaughter for future product tailoring for different markets. Non-destructive measurement of the fat content in live salmon would also make it possible to select salmon for breeding from the fat content in the muscle tissue, besides other quality factors such as increased harvest weight. No such measurements on live salmon have been reported so far.

In the present study, two different NIR instruments were used for measurement of the fat content in live salmon.

EXPERIMENTAL

In June 2001, 100 salmon from an Atlantic salmon farm (AquaGen, Kyrksæterøra, Norway) were taken individually from the sea pen. The salmon were transferred to a vessel containing 12 °C seawater with 200 mg kg^{-1} Metacainum® (MS 222, *m*-aminobenzoic acid ethyl ester methanesulphonate; Tamro, Copenhagen, Denmark). The salmon were anaesthetised within 2 min. They were first analysed using a fibre optic interactance, moving grating monochromator (FOG) instrument, with a total handling time of about 60 s, and then using a non-contact, diffuse reflectance, fixed grating, diode array (NCDA) instrument, with a handling time of 15 s. After these NIR analyses the salmon were slaughtered, bled, gutted and stored in ice water for 44 h until they had passed rigor. The analyses with the NIR instruments were then repeated. Finally, the left side of the 'Norwegian quality cut' (NQC; Fig 1), corresponding to the flesh

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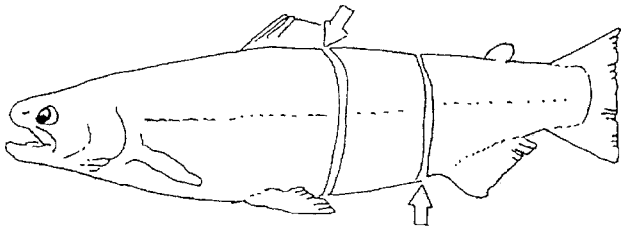

Figure 1. A salmon showing the 'Norwegian quality cut' used for determination of fat by the reference method. The left-side flesh was cut from the posterior of the backfin (upper arrow) to the gut (lower arrow).

from the backfin to the gut, was cut off for determination of the fat content by ethyl acetate extraction in five replicates.[10]

The FOG NIR instrument (NIRSystem 6500, FOSS NIRSystem, Hillerød, Denmark) was connected to a hand-held interactance probe (NR-6770-A, FOSS NIRSystem). The probe consisted of two 0.9 m long fibre optic bundles, one for guiding monochromatic light to the probe head, and one for guiding the emitted light to a silicon detector. In the probe head the bundles were split into seven parallel alternating emitting and receiving arrays, comprising a square sample area of 4.8 cm^2. The probe was mounted in a 4 cm × 4 cm metal block, and an additional black rubber collar (10 cm × 10 cm) was mounted around the probe head to shield the receiving fibres from stray light. Each apparent absorbance spectrum was the average of 20 scans in the range 400–1100 nm with 2 nm steps, giving a 24 s measurement time. Before measurement the surface of the salmon was wiped with a piece of tissue paper, then the salmon was covered with a thin plastic film (0.2 mm thick PVC foil), before the probe was placed in the middle of the NQC, 1 cm above the lateral line, on the left side of the salmon. The fibre optic probe was placed with the ribs parallel to the length direction of the salmon.

The NCDA NIR instrument (DA 7000 Flexi-mode, Perten Instrument, Huddinge, Sweden) was used in the 'up-view' mode, whereby the salmon was placed directly on the platform on a circular glass plate (diameter 125 mm). The sample was illuminated with white light. After diffuse reflection against the sample the light was diffracted through a stationary grating and split against two diode arrays, one silicon array covering 400–950 nm and another InGaAs array covering 950–1700 nm. Each apparent absorbance spectrum was the average of 1800 scans in the range 400–1700 nm with 5 nm steps, and 3 s scanning time. The salmon was placed with its left side against the circular glass plate so that the area from the backfin to the gut of the salmon, corresponding to the NQC, was illuminated. With the NCDA instrument the measurements were repeated three times on each salmon to determine the repeatability.

Partial least squares (PLS) regression was used for calibration and validation.[11] To evaluate the calibration models, full cross-validation was applied. The salmon used for each validation was left out of the data set in turn and its chemical composition was predicted by a model made from the remaining data set. The multivariate prediction correlation coefficient and the prediction error expressed as the root mean square error of prediction (RMSEP) were used as predictive quality criteria. The RMSEP is defined as

$$\mathrm{RMSEP} = \left(\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{N} \right)^{1/2} \quad (1)$$

where y_i and $\hat{y}_i$ are the reference method and NIR-predicted fat concentrations respectively for each sample i (1,2,...,N).

The reference method precision for the fat measurements was calculated using the standard error of the reference method (S_{ref}), defined as

$$S_{\mathrm{ref}} = \left(\frac{\sum_{i=1}^{N} S_i^2}{NM} \right)^{1/2} \quad (2)$$

where S_i is the standard deviation of M replicates for each sample i (1,2,...,N).

Regression analysis was performed using UNSCRAMBLER version 7.6 (Camo ASA, Oslo, Norway).

RESULTS AND DISCUSSION

Physical and chemical measurements

Table 1 gives an overview of the physical and chemical measurements. The body weight and length of the salmon showed a clear but non-linear correlation (Fig 2). The larger salmon also had a higher fat content, but the size of the salmon did not give a satisfactory estimate of the fat content (Fig 3). Salmon with a body weight below 4 kg contained between 80 and 190 g kg^{-1} fat in the flesh, while above 4 kg body weight the fat content varied between 160 and 230 g kg^{-1}.

NIR spectra

Fig 4 shows the average absorbance spectra taken with the FOG instrument for the live salmon and for the same salmon post-rigor. The spectrum from the live salmon shows higher absorbance than that from the post-rigor salmon. The same is also seen for the

Table 1. Range, mean value (100 fish) and standard deviation (SD) of fat content and physical characteristics of salmon

	Range	*Mean*	*SD*	S_{ref} [a]
Fat content (g kg^{-1})	82.0–231.6	173.4	31.7	0.6
Body weight (kg)	0.83–11.10	4.77	3.00	
Carcass weight (kg)	0.73–10.40	4.32	2.74	
Length (cm)	43.5–92.0	68.6	14.14	

[a] Standard error of reference method, eqn (2).

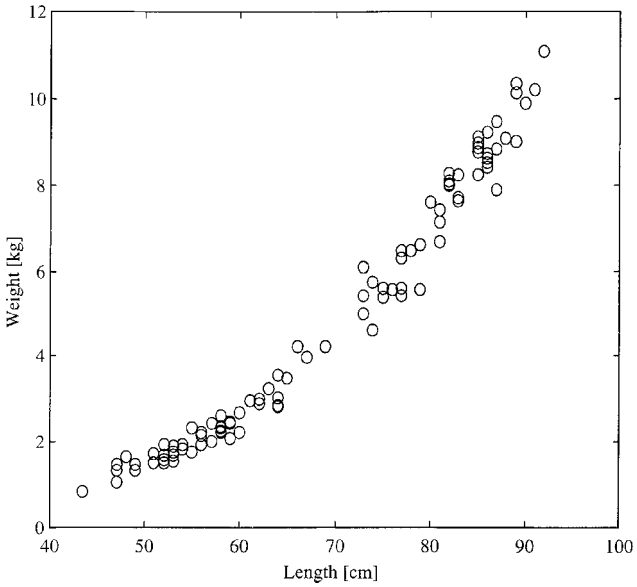

Figure 2. Whole salmon weight as a function of length.

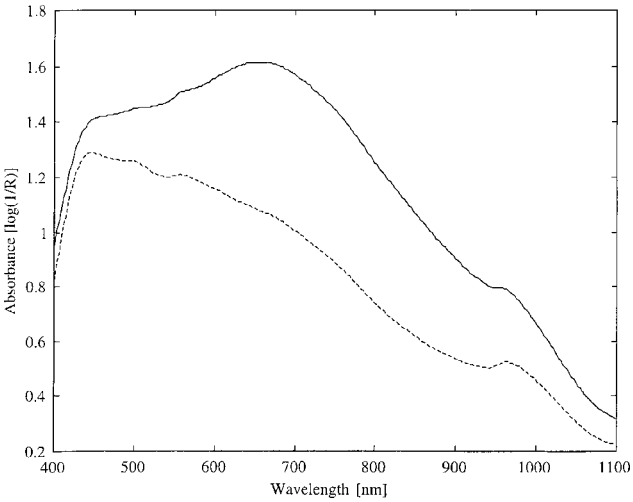

Figure 4. Average spectra of live (full curve) and post-rigor (broken curve) salmon measured by the fibre optic interactance probe and moving grating NIR instrument (FOG).

corresponding spectra taken with the NCDA instrument (Fig 5). This absorbance difference is caused by higher scattering of diffuse light in the post-rigor salmon. This is probably due to protein denaturation, giving more internal surface area for light scattering. Live salmon flesh is more transparent than post-rigor salmon flesh. The temperature difference (about 12 °C) between the live and post-rigor salmon is expected to lead to only minor spectral differences compared with the effects of physiological state.

For the FOG instrument the visible range of the spectrum (400–800 nm) showed higher absorbance than the NIR region (800–1100 nm) (Fig 4). This was because the probe was held only against the dark part of the skin. With the NCDA instrument the absorbance in the visible range of the spectrum did not increase with increasing wavelength, because it is an average of the whole illuminated area consisting of both the upper dark skin and the lower silver-coloured skin (Fig 5). The diffuse reflectance from the visible range of the spectrum reflects information about the colour of the skin, and it was found necessary to delete the visible part of the spectrum from the PLS calibration.

NIR calibrations

The optimal wavelength range was found to be 800–1098 nm for the FOG instrument and 900–1700 nm for the NCDA instrument.

The RMSEP decreased somewhat faster for the NCDA instrument with increasing number of PLS factors: but the overall RMSEP values were similar for the two instruments (Fig 6). The correlation coeffi-

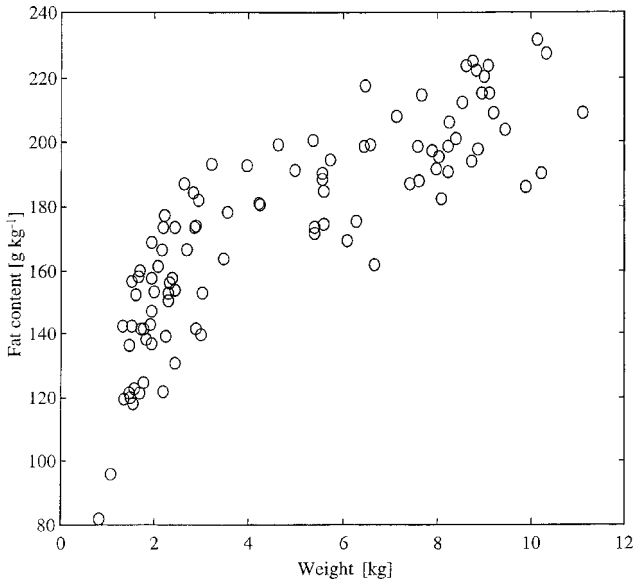

Figure 3. Fat content as a function of whole salmon weight.

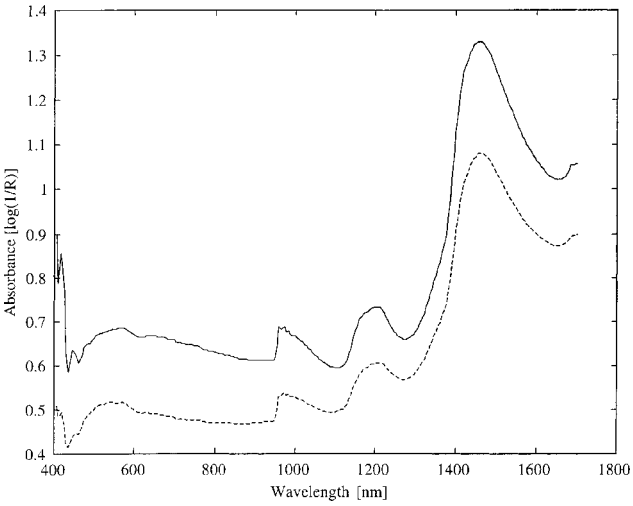

Figure 5. Average spectra of live (full curve) and post-rigor (broken curve) salmon measured by the non-contact reflectance diode array NIR instrument (NCDA).

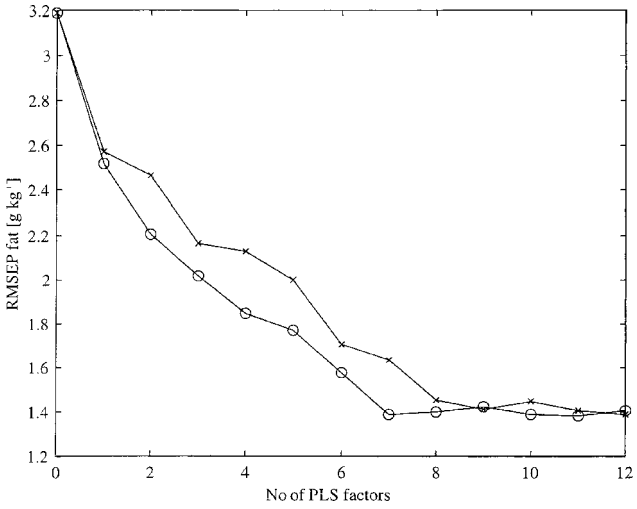

Figure 6. Prediction error, expressed as root mean square error of prediction (RMSEP), of fat as a function of number of PLS factors for (crosses) the fibre optic interactance probe and moving grating NIR instrument (FOG) and (circles) the non-contact reflectance diode array NIR instrument (NCDA).

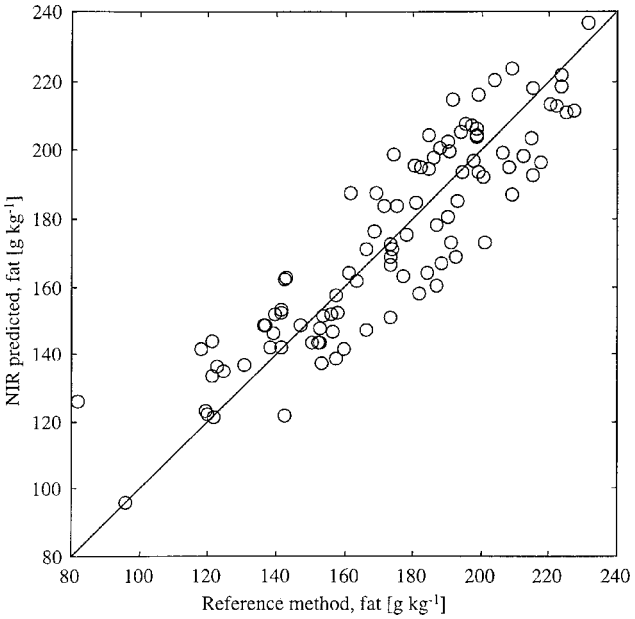

Figure 7. NIR-predicted (cross-validation) *versus* reference method fat content of live salmon. NIR was measured using the fibre optic interactance probe and moving grating instrument (FOG). The diagonal line is the target line. The correlation coefficient is 0.90.

cient between measured and predicted values for the live and post-rigor salmon was about 0.90 for both instruments (Table 2). The predictive performance for the live salmon was similar for the two instruments, with an optimal RMSEP of about 14 g kg^{-1}. The predictive performances for the live salmon are shown in Figs 7 and 8 for the FOG and NCDA instruments respectively. The performance for the post-rigor salmon was somewhat improved with the FOG instrument but not with the NCDA instrument. However, the NCDA measurement was always performed after the FOG measurement. Before the NCDA measurement the thin plastic foil covering the surface of the salmon during the FOG measurement was removed, resulting in an uneven slime on the surface of the salmon. Therefore the surface was wiped again with tissue paper before the NCDA measurement. This handling resulted in slight damage to the skin. It was shown previously that scraping off the scales increased the prediction error.[8] Thus the difference in the prediction error in our work may be explained by this slight damage to the skin. Earlier experiments with the NCDA instrument on post-rigor whole salmon gave an RMSEP of 11 g kg^{-1} fat.[9] Similar results have been reported for FOG measurements of post-rigor salmon.[6,8] In these earlier reports the range of salmon weights was considerably smaller (1–6 kg) than in the present experiment (1–11 kg), which could explain our increased prediction error.

Attempts to predict the fat content of live salmon using a post-rigor calibration model resulted in a strongly increased RMSEP and a bias of the regression

Table 2. Predictive performance of FOG (800–1098 nm) and NCDA (900–1700 nm) instruments; 100 samples were analysed live and post-rigor, giving 200 measurements in the combined prediction model for live and post-rigor salmon

Instrument	*Status*	*Number of PCs*	*Correlation coefficient*	*RMSEP (g kg^{-1})*
FOG	Live	9	0.90	14.1
NCDA	Live	7	0.90	13.9
FOG	Post-rigor	7	0.91	12.9
NCDA	Post-rigor	6	0.88	15.0
FOG	Live + post-rigor	7	0.91	12.9
NCDA	Live + post-rigor	10	0.90	14.0

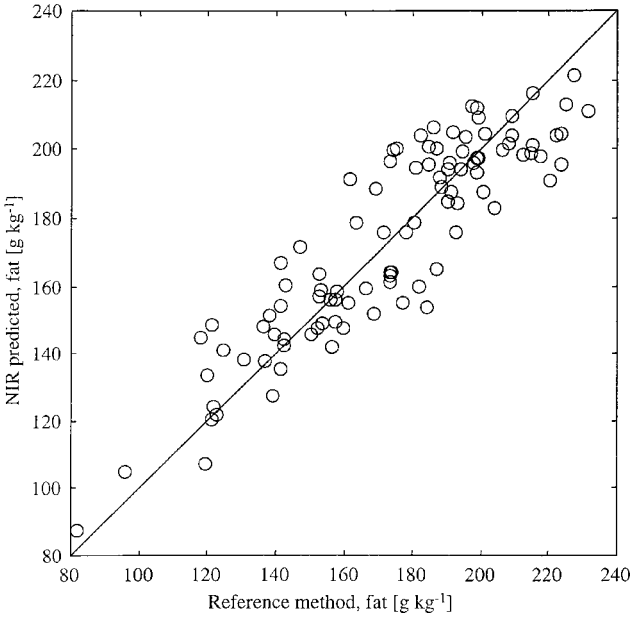

Figure 8. NIR-predicted *versus* reference method fat content of live salmon. NIR was measured using the non-contact reflectance diode array instrument (NCDA). The diagonal line is the target line. The correlation coefficient is 0.90.

Table 3. Prediction of fat content from NIR measurement of 100 live salmon with a calibration model from 100 post-rigor salmon

Instrument	Correlation coefficient	RMSEP ($g\,kg^{-1}$)	Bias ($g\,kg^{-1}$)
FOG	0.85	43.5	−40.3
NCDA	0.85	21.7	−12.7

line (Table 3). However, when a combined calibration model consisting of all 200 measurements of the live and post-rigor salmon was made (Table 2), there was no bias and the NIR measurement could be used to predict the fat value for the salmon independently of the rigor status.

The NIR analysis was repeated three times on the NCDA instrument. The variation in the predicted values was $\pm 1\,g\,kg^{-1}$ fat (data not shown). This high repeatability compared with the 10-times-larger prediction error indicates that the measurement time could be reduced from 3 to 1 s. In a modern salmon slaughterhouse, 20–30 salmon will be processed per minute. An NCDA instrument should be fast enough to allow on-line sorting of individual salmon according to their fat content, requiring 1 s for orienting the salmon on the measuring plate and 1 s for the analysis. When an organic anaesthetic such as Metacainum® has been used, the salmon has to recover and live for additional three weeks before slaughter for human consumption. If one wants to sort alive salmon in a slaughterhouse, the salmon has to be immobilised in other ways, such as cooling down the fish below 4 °C.[12,13] During selection of salmon for breeding, the measurement time is less critical and either instrument could be used, especially if the salmon are anaesthetised during the sorting. It is, in our knowledge no restriction to use a salmon for breeding short time after an anaesthetisation.

CONCLUSIONS

NIR measurement, either with the FOG instrument or with the NCDA instrument, could be used as a screening method to determine the crude fat content in live farmed Atlantic salmon. It is necessary to have measurements from live salmon in the calibration model in order to get a good prediction for live salmon. The predictive performances were similar for the two instruments, but the NCDA instrument was much faster, making it more suitable when large numbers of samples have to be sorted. The results shows that NIR is a method for sorting live salmon according to their fat content. By measuring the fat content during the feeding period, product tailoring for different markets is now possible. It will also be possible to select salmon for breeding from the fat content in the muscle tissue, besides other quality factors such as increased harvest weight.

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